Structure *14*, August 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.str.2006.07.003

Gaining Access to ERp57 Function

ERp57, in complex with the lectins calreticulin and calnexin, catalyzes disulphide bond formation in N-glycosylated proteins. In this issue of *Structure*, Kozlov et al. (2006) report the structure of the noncatalytic domains of ERp57 and characterize the calnexin interaction site.

Native disulphide bond formation in the endoplasmic recticulum (ER) is complex, occurring via multiple parallel pathways. While some of the participants in this cellular process are known, their individual roles are still largely confused. However, the rate-limiting folding steps for proteins that contain multiple disulphides are late-stage isomerization reactions, where disulphide bond formation is linked to conformational changes in protein substrates with substantial regular secondary structure, and these reactions are catalyzed only by members of the protein disulphide isomerase (PDI) family.

PDI family members differ considerably between different organisms. There are at least 17 human PDI family members (Ellgaard and Ruddock, 2005), most of which are poorly characterized with little or nothing being known about their physiological function(s). Bovine PDI was the first protein folding catalyst reported over 40 years ago (Goldberger et al., 1963), but significant details of the mechanisms of action of the PDI family are still unknown. In part, this is due to the complexities of its activities and substrates, but work in the field has also been severely hampered by the inability for over 30 years to crystallize catalytically active PDI family members or multidomain constructs. Very recently the first structure of a PDI was published, Pdi1p from *S. cerevisiae*, which revealed that each of the four domains, **a**, **b**, **b**′, and **a**′, has a thioredoxin fold (Tian et al., 2006). This 2.4 Å structure gave important insights into the domain organization of this protein and will undoubtedly affect many functional studies. Following hard on the heels of that study, the group of Kalle Gehring and coworkers has solved the structure of the noncatalytic **bb**′ domains of ERp57 to 2.0 Å (Kozlov et al., 2006).

After PDI itself, ERp57 is the most widely studied PDI family member. It is thought to be dedicated to the folding of N-glycosylated proteins, with the specificity for substrates arising through its interactions with the ER-resident lectins calreticulin (CRT) and calnexin (CNX). In their detailed and extensive study, Kozlov and coworkers have not only solved the crystal structure of the **bb**′ domains of ERp57 (Figure 1), but they have also obtained information on full-length ERp57 via small-angle X-ray scattering (SAXS; Kozlov et al., 2006). Both domains unsurprisingly adopt a thioredoxin fold, as seen in Pdi1p (Tian et al., 2006), but there are important differences between the two structures, not least the orientation of the domains with respect to each other. However, it is unclear if this is an ERp57-specific effect or if crystal packing plays a role, especially since the Pdi1p structure appears to have the **b** domain of one Pdi1p molecule located in the substrate binding groove formed between the four domains of another Pdi1p molecule (Tian et al., 2006). ERp57 also contains two insertions in the **b** domain, including an enlarged β4-β5 loop, which plays a role in CNX interactions (see below), while the **b**′ domain of Pdi1p contains a significant insertion between β5-α4 of as yet unknown function. Looking at the full protein, SAXS data implies that the average conformation of ERp57 in solution closely resembles that of Pdi1p (Kozlov et al., 2006; Tian et al., 2006).

NMR and ITC studies (Kozlov et al., 2006) allowed the identification of the CNX interaction site on ERp57 as a conserved positively charged region on the **b**′ domain, along with spatially adjacent residues from the enlarged β4-β5 loop in the **b** domain. These studies also implicated a conserved negatively charged region at the tip of the P domain of CNX in the CNX-ERp57 interaction, results which resemble those previously reported for CRT-ERp57 interactions (Frickel et al., 2002). While the association between CNX and ERp57 is primarily electrostatic in nature, other factors must also participate, since a single point mutation (M347A) in CNX also completely abrogated the interaction (Kozlov et al., 2006).

Recently, the ERp57 knockout was described. While the mouse knockout was embryonic lethal, consistent with the key role in N-glycoprotein folding and quality control reported for ERp57 (reviewed in Ellgaard and Helenius, 2003), the results from knockout cell lines showed remarkably little phenotype (Garbi et al., 2006; Solda et al., 2006). This lack of phenotype probably results from functional redundancy between the 17 human PDI family members, specifically the potential for ERp72 to replace ERp57 (Ellgaard and Ruddock, 2005). When mapped onto the structure of ERp57 **bb**′, sequence conservation between ERp57 and ERp72 looks similar to that reported for the conservation of surface residues in ERp57, including in the CNX interaction site (see above). Hence, it is likely that ERp72 can interact with CRT/CNX under normal physiological conditions as well as in the ERp57 knockout cell line (Solda et al., 2006).

The mechanism of action of the PDI family as catalysts of native disulphide bond formation in polypeptide folding requires the ability to catalyze disulphide-dithiol exchange and to bind nonnative proteins. While ERp57 almost certainly has substrate binding sites in the catalytically active **a** and **a**′ domains as per PDI (Koivunen et al., 2005), the primary substrate specificity for protein substrates arises through its interaction with CRT/CNX, which in turn bind monoglucosylated protein substrates. CNX binding by ERp57 is critical for ERp57-catalyzed refolding of N-glycosylated substrates, but it does not stimulate the reduction of di(o-aminobenzyl)-labeled oxidized glutathione by ERp57, suggesting that CNX/CRT serves to bind and position substrates rather than directly stimulating the oxidoreductase activity of ERp57 (Kozlov et al., 2006). However, the unusual elongated structure of the P domain of CNX/CRT (Schrag et al., 2001; Ellgaard et al., 2001) and the positioning of the CNX/CRT binding site on the opposite face of ERp57 relative to the catalytic thiols (Figure 1A) offer another

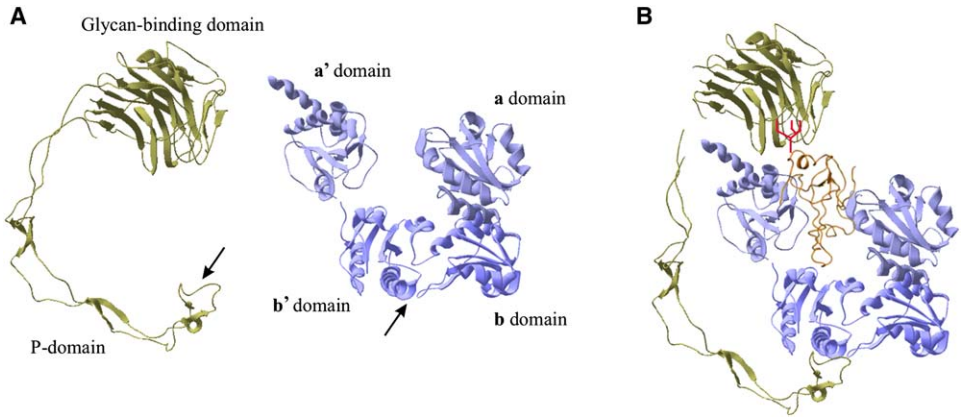

Figure 1. CNX-ERp57 Interactions May Promote Folding

(A) Structure of CNX (Schrag et al., 2001) and ERp57 (constructed from the structures of ERp57 **bb**′ and the **a** and **a**′ domains of Pdi1p [Tian et al., 2006] as per Kozlov et al. [2006]), with the mutual interaction sites (Kozlov et al., 2006) indicated with arrows. CNX is shown in yellow, and ERp57 in blue. The ERp57 **bb**′ coordinates (Kozlov et al., 2006) were generously communicated by the authors prior to publication.

(B) Schematic of the CNX-ERp57-substrate complex. Note that the orientation of CNX with respect to ERp57 is currently unknown. ERp57 binds nonnative protein substrates and forms mixed disulphides with the substrate via its catalytic **a** and/or **a**′ domains, while CNX binds the monoglucosylated N-glycan (Glc_1-Man_9-$GlcNAc_2$, shown stylized in red) via its glycan binding domain. Any movement of CNX relative to ERp57, for example, flexing of the elongated hairpin P domain or of the **a** and **a**′ domains of ERp57 relative to each other, will result in conformational changes in the bound substrate. Such motions could potentially allow ERp57 access to buried cysteines and/or disulphides.

interesting mechanistic possibility. Since PDI family members have to access buried thiol residues in polypeptide substrates with substantial regular secondary structure, the action of ERp57 with CNX/CRT must, in some way, be able to trigger conformational changes in bound substrate. As CNX/CRT and ERp57 have a limited interaction site far removed from their substrate binding sites (Figure 1B; Kozlov et al., 2006), there is the potential for the movement of the substrate binding sites of CNX/CRT and ERp57 with respect to each other. Any such movement of CNX/CRT with respect to ERp57 could deform the mutually bound N-glycosylated polypeptide substrate, especially if, like most substrates for ERp57, it lacks stable tertiary structure. Such speculation requires either structural or modeling studies on the dynamics of the interaction between CNX/CRT and ERp57, something which the Kozlov et al. (2006) paper brings into reach.

Lloyd W. Ruddock[1]
[1] Department of Biochemistry and Biocenter Oulu
University of Oulu
P.O. Box 3000
FIN-90014 Oulu
Finland

Selected Reading

Ellgaard, L., and Helenius, A. (2003). Nat. Rev. Mol. Cell Biol. *4*, 181–191.

Ellgaard, L., Riek, R., Herrmann, T., Gunert, P., Braun, D., Helenius, A., and Wüthrich, K. (2001). Proc. Natl. Acad. Sci. USA *98*, 3133–3138.

Ellgaard, L., and Ruddock, L.W. (2005). EMBO Rep. *6*, 28–32.

Frickel, E.M., Riek, R., Jelesarov, I., Helenius, A., Wüthrich, K., and Ellgaard, L. (2002). Proc. Natl. Acad. Sci. USA *99*, 1954–1959.

Garbi, N., Tanaka, S., Momburg, F., and Hämmerling, G.J. (2006). Nat. Immunol. *7*, 93–102.

Goldberger, R.F., Epstein, C.J., and Anfinsen, C.B. (1963). J. Biol. Chem. *238*, 628–635.

Koivunen, P., Salo, K.E.H., Myllyharju, J., and Ruddock, L.W. (2005). J. Biol. Chem. *280*, 5227–5235.

Kozlov, G., Maattanen, P., Schrag, J.D., Pollock, S., Cygler, M., Nagar, B., Thomas, D.Y., and Gehring, K. (2006). Structure *14*, this issue, 1331–1339.

Schrag, J.D., Bergeron, J.J., Li, Y., Borisova, S., Hahn, M., Thomas, D.Y., and Cygler, M. (2001). Mol. Cell *8*, 633–644.

Soldà, T., Garbi, N., Hämmerling, G.J., and Molinari, M. (2006). J. Biol. Chem. *281*, 6219–6226.

Tian, G., Xiang, S., Noiva, R., Lennarz, W.J., and Schindelin, H. (2006). Cell *124*, 61–73.